DISCUSSION

Graft rejection is a serious complication after renal transplantation. In fact, the rejection episode can be prevented or at least more effective treated, if they are detected earlier rather than later. The early recognition of acute rejection and prompt treatment may completely reverse and minimize the unfavorable effects on long-term graft function. Nonetheless, the early detection of acute renal rejection after successful transplantation is difficult and invasive procedure such as protocol graft biopsies may be required. Various factors influence posttransplant complications including the immune status of the recipients and the effects of immunosuppressive drugs[33]. The calcineurin inhibitors, cyclosporine and tacrolimus, are drugs commonly used to prevent and to treat rejection episode of renal allograft. Clinical use of these drugs is complicated since immunosuppressive drugs have the narrow therapeutic index, and the variable and unpredictable pharmacokinetics. There can be large fluctuations in the drug levels between individual patients (interindividual variation) and within the same patient (intraindividual variation) receiving the same dose of drug[6,34]. Subtherapeutic drug levels are correlated with episodes of graft rejection while higher than therapeutic drug levels tend to decrease graft function due to drug-induced nephrotoxicity. Furthermore, the toxicity of calcineurin inhibitors on renal dysfunction can occur without warning symptoms of abnormalities[33]. As a result, it is generally not possible to determine the correct dose of these immunosuppressive drugs without monitoring the blood levels. The ranges of therapeutic drug concentrations are different depending on the time after transplantation and the use of concomitant drugs. Generally the range of therapeutic drug levels for the first 2-6 months is rather higher than 6 months after transplantation. The reason may be due to a decrease in risk of rejection after 6 months of successful graft transplantation, consequently, the therapeutic range of a drug decreases and a dose of a drug can be reduced to maintain the drug level within the lower therapeutic range[2,6].

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Hyaluronan is a principal ligand of CD44. CD44 is an important leukocyte cell surface glycoprotein with diverse functions including cell adhesion, homing, migration, and activation. The interaction of HA and CD44 mediates the recruitment of inflammatory cells, the earliest events in an inflammatory response, and also promotes granulocyte phagocytosis of apoptotic bodies as well as cell-mediated cytotoxicity in natural killer cells[35]. *In vitro,* both cortical and medullary fibroblast of kidneys are capable of synthesizing HA. This synthesis is enhanced by various growth factors, which are produced in increased amounts in inflammation[36]. Although little HA level is found in normal kidney, several studies have shown up-regulated expression of HA in tubulointerstitial injury, glomerulonephritis, and rejection of human kidneys[13]. Lymphocyte activation and infiltration into a transplanted organ is a major component of the rejection process. Graft infiltration of lymphocytes requires adhesion of leukocytes to the endothelium, diapedesis, and transmigration. The important protein involved in this process is CD44, which interacts with endothelial HA. As a result, HA may be an important mediator in response to tissue injury and graft rejection. It should be presented at high concentrations to act as a support for cell adhesion and locomotion. Since the HA levels and the immunosuppressive drug levels are associated with graft status in renal transplant recipients, the finding of their relationships would be useful to predict rejection episode and/or prognosis of graft status as well as to detect the adverse reactions of the immunosuppressive drugs.

This study determined the HA levels from blood samples that were obtained from 101 renal transplant recipients sending the samples for immunosuppressive drug monitoring. The average HA level was within normal range of 20.06±12.90 (range:0.38-135.78) ng/ml and was not significant difference when compared with control [N=22, 18.38±2.89 (range:5.43-19.00) ng/ml]. The higher HA levels of > 100 ng/ml were found in 3 samples, however, were not correlated to any rejection criteria and immunosuppressive drug levels. Statistical analysis could not find significant correlation between HA levels and immunosuppressive drug levels; cyclosporine and tacrolimus. The reason may be due to a successful renal transplantation and patients were within the good condition that could maintain their graft status. Because of this, the HA levels would be within normal range despite a wide variations in immunosuppressive drug levels. This study could not showed the values of the HA levels of the patients before renal transplantation, which were expected to be higher than normal values. However, we have determined the HA levels

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from patients with end-stage renal disease (N=68), who had normal liver function and stayed alive by weekly hemodialysis. The result showed that mean HA level in end-stage renal disease was 146.25±291.48 (range: 0.46-2,249.48) ng/ml. We expected that if these patients received the successful renal transplantation, the HA levels would return to normal range. The previous study has also demonstrated high HA levels in patients whose clinical condition deteriorated before renal replacement therapy and the levels return to normal after successful renal transplantation[14].

Another reason may be due to a rapid elimination of HA, with a half-life of 2-6 minutes, and a total turnover rate of 10-100 mg/d. The main elimination of circulatory HA is by receptor-mediated endocytosis in hepatic sinusoidal endothelial cells and the fast turnover rate in turn, affect the circulatory HA level[10,26,27]. The level of HA is also dynamic and several factors such as diet, posture, activity, or clinical deviation, can influence the HA level to a different extent[9]. In case of inappropriate immunosuppressive drug levels or graft rejection, the level of HA may rise rapidly in response to immuno-stimulation or inflammation. However, since the liver of renal transplant recipient was not impaired, it could eliminate HA very efficiently, thus, resulted in a lower than expected value of HA levels.

The effect of immunosuppressive drug on the production of HA may influence the levels of HA. Cyclosporine inhibits T-cell activation as well as prevents the induction of genes encoding for cytokines and their receptors. Thus, humoral and cellular immune responses are abolished, resulting in the successful graft acceptance. Cyclosporine may have other mechanisms that might be involved in different functions of the immune response such as preventing the transcription of a group of genes, concomitantly inducing the transcription of another set, which may suppress the production of HA. Similarly, tacrolimus exerts its effects through impairment of gene expression in target cells. This drug has various actions such as inhibits calcium-dependent events, interleukin-2 gene transcription, nitric oxide synthase activation, cell degranulation, and apoptosis. Tacrolimus also potentiates the actions of glucocorticoids by binding to FKBPs contained within the hormone receptor complex, and thus preventing degradation. The agent may enhance expression of the transforming growth factor beta-1 gene in a fashion analogous to that demonstrated for cyclosporine. Type 1 T-helper cells appear to be preferentially suppressed compared with type 2 T-helper cells. T-cell mediated cytotoxicity is impaired. B cell growth and antibody production are affected indirectly by the suppression of T cell-derived growth factors

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necessary for these functions. As mention earlier, in case of renal allograft rejection, the HA production may come from several cells such as renal interstitial fibroblasts of the cortex and renal medulla. The etiology of increased production of HA may be due to the inflammation and immuno-stimulation depended on clinical status of the patients. The immunosuppressive drugs, which play the important role in the immune response, may also inhibit the production of HA. In addition, if the HA is produced, it will remain or accumulate within the kidney tissue. The release of HA from the kidney may be less than other cells and since its elimination is so rapid that influence on the rather low level of HA in renal transplant recipients. Moreover, the reason may be due to the role of CD44-HA interactions, which are important in the recruitment of inflammatory cells, but are not necessarily critical in the pathogenesis of rejection[16].

The lack of relationship between HA level in the blood and rejection episode is not in agreement with previous studies that have reported the increased in HA amounts in the rejecting kidneys[11-13,15-19]. Interestingly, most of the rejected kidneys have been studied in the core graft biopsies, which are important especially for the poor kidney function of renal transplant patients, and promptly perform only in case of the unsatisfactory response to high-dose immunosuppressive treatment[12,13,33]. In addition, the interstitial HA concentration is usually one to two orders of magnitude higher than the circulatory HA[9]. Therefore, further study of HA level as a prognostic marker in renal transplant patients should be done in the core biopsy of the renal specimens.

The limitation of this retrospective study is an unable to collect the HA level before graft transplantation. The level of HA before transplantation could be use as a basal HA level and after successful transplantation would result in the low level if the HA level could be use as a prognostic marker. Moreover, the nature of population in this study was the population with successful therapy, thus the HA levels were within normal range and the HA could not be use as a tool prognosis for monitoring rejection. In addition, the time of sampling is the most important. When suspected episode of acute rejection, the blood should be drawn for HA levels immediately. The sampling time in this study was a time for drug monitoring, therefore, inappropriate to determine the HA levels. Finally, the HA levels from the blood and the core biopsy of kidney should be determined concurrently to answer whether they could represent each other. Further study regarding HA levels in renal transplanted recipients is still mandatory.

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